IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS : Lee *et al.*
SERIAL NUMBER: 10/027,186 EXAMINER : Goldberg, Jerome D.
FILING DATE : December 20, 2001 ART UNIT : 1614
FOR : METHODS FOR MODULATING TUMOR GROWTH AND METASTASIS

DECLARATION UNDER 37CFR§1.132

I, Dr. Sally Hill, of the Gray Cancer Institute, London , United Kingdom declare and state that:

1. I am presently a Senior Scientist in the Tumor Microcirculation Group at the Gray Cancer Institute in London, United Kingdom. I am a specialist in tumor biology and have conducted research in cancer therapy for the past 30 years. I obtained a doctorate in radiation biology from the University of London in 1980, and have been conducting research as a member of the Gray Cancer Institute since 1973.

2. I have performed, or have had performed under my supervision, studies evaluating the properties of Combretatastin A-4 Phosphate (hereinafter, "CA4P"). These studies have been conducted in collaboration with scientists at OXiGENE, Inc., a co-assignee of the above-referenced patent application.

3. I have read and analyzed the data supplied in the subject application and the references cited by the examiner. I have reviewed the pending claims of the above-referenced application (see **Appendix I**) which I understand are drawn to combination chemotherapy for treating tumor growth or metastasis in a subject suffering from cancer. The combination chemotherapy involves the administration of a combination or "cocktail" of chemotherapeutics that includes CA4P together with Paclitaxel (also known as Taxol).

4. I have been informed and understand that the pending claims of the above-referenced application are rejected as being obvious over Pettit (US Patent No. 5;561,122) in combination with an abstract from Cahan *et al.*, (1994) Cancer Chemotherapy and Pharmacology, 33(5): 441-5. The Pettit reference describes CA4P as a potent inhibitor of tumor cell growth *in vitro*. Cahan *et al.* teaches that Taxol has exhibited clinical activity in the treatment of certain tumors.

5. The methods of the invention have been repeated in my laboratory and under my supervision. It is my opinion that the use of a CA4P + Taxol drug cocktail as currently claimed in the present application leads to superior and unexpected results that render the claims unobvious and patentable over the prior art,

including the aforementioned references. For example, the simultaneous administration of CA4P and Taxol results in an inhibitory effect on tumor cell viability that is not merely a result of the additive effects of the individual properties of these agents. Rather, a genuine synergistic effect (*ie.* a greater than additive effect) on tumor cell survival is achieved by combining these agents. Moreover, the methods claimed by the Applicants are quite unexpected in light of the inherently antagonistic and opposing mechanisms of action of each agent.

6. Prior to observing the synergistic effects of the Applicant's invention, we had conducted extensive experiments to evaluate the anti-tumor properties of CA4P (see **Appendix II**). In our standard CaNT mouse model of an adenocarcinoma tumor, we observed a mean tumor cell survival fraction of 0.134 (*i.e.* ~87% tumor cell kill). These results indicated that, despite the potent anti-tumor activity of CA4P, a substantial fraction of viable tumor cells remained to repopulate the tumor and contributed to its continued growth.

7. To evaluate the anti-tumor effects CA4P + Taxol combination therapy in our model, an experiment was conducted in which the same ineffective dose of CA4P (50mg/ml) was combined with a maximum tolerable dose of Taxol. The following groups of CBA/Gy f TO mice bearing a murine adenocarcinoma NT (CaNT) tumor were administered the indicated treatments:
   Group A: Saline-only control
   Group B: Taxol (30 mg/ml)
   Group C: Taxol (30 mg /ml) + CA4P (50 mg/ml)
   (Note: Group C animals were administered CA4P and Taxol simultaneously)

8. An *in vitro* clonogenic (colony-forming) assay was performed to assess the survival of tumor cells excised from each treatment group. The results of tumor cell survival following colony growth for 3 separate experiments are tabulated in Table 1 (see Appendix II). Data for Groups B and C were normalized with respect to Group A and expressed as mean surviving fraction of tumor cells per gram of tumor ("S.F./g") for each treatment group. The results clearly demonstrate a synergistic effect of combining Taxol with CA4P. Administering Taxol alone resulted in only a 64% (S.F./g = 0.3574) reduction of viable tumor cells. By contrast, administering Taxol in combination with CA4P resulted in a 97.7% reduction (S.f./g = 0.0233) in tumor cell survival. This nearly complete tumor cell kill can result in a considerable delay in the growth of a tumor mass (see Figure 1 of Appendix II. Note: Results are summarized from a separate experiment evaluating tumor growth control).

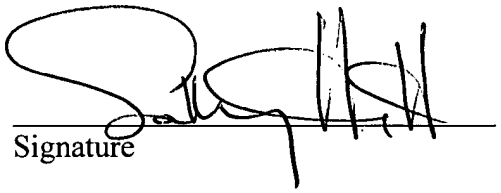
9. Based on the anti-tumor effects observed for each individual agent, an additive effect would have provided a surviving fraction of 0.048 (*i.e.* 0.36 X 0.134 = 0.048) or a 95.2% reduction in a tumor cell viability. Since the amount of cell kill observed with the CA4P + Taxol combination (0.0233) was over a factor of two greater than the amount of cell kill previously obtained with CA4P alone, it was concluded that the results obtained with the CA4P + Taxol combination were not

merely an additive effect of the combination but a greater than additive (*ie.* synergistic) effect.

10. The above-described synergistic properties specifically associated with the CA4P + Taxol combination therapy were unexpected in view of the mechanisms of action that are commonly accepted for each agent. Taxol is known to exert its effects on tumor growth control by binding to polymerized microtubules of a rapidly proliferating tumor cell and stabilizing them, thereby inhibiting their de-polymerization and arresting tumor cell division [Gelmon K., The Lancet, (1994); 344: 1267-1272]. In contrast, CA4P has been ascribed an opposing mechanism of action in which binding of the agent to β-tubulin monomers (as opposed to polymerized tubulin) prevents their assembly into microtubules [Sackett D., Pharmacol Ther., (1993); 59(2):163-228]. Accordingly, those knowledgeable in the art of anti-cancer therapy at the time of the invention would have expected CA4P to antagonize the effects of Taxol and vice versa.

11. In conclusion, the discussion above demonstrates that the CA4P + Taxol combination therapy claimed in the present application provides unexpected and superior results compared to single-agent therapies that employ either agent alone. Moreover, these results expand the clinical usefulness of both agents. Accordingly, it is my opinion that the subject matter claimed in the present application is unobvious over the prior art.

12. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C §1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.



Signature

Signed at 15.30

this 17th day of Nov., 2004.

APPENDIX I
(CLAIMS AS CURRENTLY PENDING)

1. (Original) A method for modulating tumor growth or metastasis in an animal in need thereof, comprising sequential or simultaneous administration of at least one anticancer agent, and a combretastatin A4 compound in amounts effective therefor.

2. (Original) A method for modulating tumor growth or metastasis in an animal in need thereof, comprising administration of a combretastatin A4 compound and at least one anticancer agent, in amounts effective therefor, wherein said combretastatin A4 compound is administered at a time relative to administration of said anticancer agent sufficient to modulate blood flow to said tumor to provide a time-dependent effective tumor concentration of said anticancer agent.

3. (Previously Amended) The method as claimed in claim 1, wherein said at least one anti-cancer agent is a mitotic inhibitor.

4. (Previously Amended) The method as claimed in claim 1, wherein said at least one anticancer agent is selected from the group consisting of paclitaxel and docetaxel.

5.-6. (Cancelled)

7. (Previously Amended) The method as claimed in claim 1, wherein said anticancer agent is a duration exposure agent and is administered sequentially in any order with said combretastatin A4 compound.

8. (Previously Amended) The method as claimed in claim 7, wherein said duration exposure agent is a taxane.

9.-13. (Cancelled)

14. (Previously Amended) The method as claimed in claim 8, wherein said combretastatin A4 compound is a combretastatin A4 phosphate prodrug salt and said taxane is paclitaxel.

15. (Previously Amended) The method as claimed in claim 14, wherein said combretastatin A4 phosphate prodrug salt is administered at least 3 hours prior to paclitaxel.

16.-21. (Cancelled)

22. (Original) A pharmaceutical composition for modulating tumor growth or metastasis in an animal in need thereof, comprising at least one anticancer agent, and a combretastatin A4 compound, in amounts effective therefor in a pharmaceutically acceptable carrier.

23. (Previously Amended) The pharmaceutical composition as claimed in claim 22, wherein said at least one anticancer agent is a taxane.

24. (Previously Amended) The pharmaceutical composition of claim 23, wherein said combretastatin A4 compound is a combretastatin A4 phosphate prodrug salt.

25.-36. (Cancelled)

37. (Previously Amended) The pharmaceutical composition as claimed in claim 23, wherein said taxane is paclitaxel.

38. (Previously Amended) The pharmaceutical composition as claimed in claim 24, wherein said taxane is paclitaxel.

39.-60. (Cancelled)

61. (Previously Presented) The method of claim 14, wherein said paclitaxel is administered at least 24 hours prior to said combretastatin A4 phosphate prodrug salt.

62. (Previously Presented) The method of claim 1, comprising administration of a second anticancer agent.

63. (Previously Presented) The method of claim 62, wherein said anticancer agent is a taxane and said second anticancer agent is an alkylating agent.

64. (Previously Presented) The method of claim 62, wherein said second anticancer agent is selected from the group consisting of: cisplatin, carboplatin, and oxaliplatin.

65. (Previously Presented) The method of claim 63, wherein said taxane is paclitaxel and said alkylating agent is carboplatin.

66. (Previously Presented) The method of claim 65, wherein paclitaxel and carboplatin are administered simultaneously to each other and at least 24 hours prior to said combretastatin A4 compound.

67. (Previously Presented) The pharmaceutical composition of claim 22, comprising a second anticancer agent.

68. (Previously Presented) The pharmaceutical composition of claim 67, wherein said second anticancer agent is selected from the group consisting of: cisplatin, carboplatin, and oxaliplatin.

69. (Previously Presented) The pharmaceutical composition of claim 68, wherein said second anticancer agent is carboplatin.

70. (Previously Presented) The pharmaceutical composition of claim 37, comprising a second anticancer agent.

71. (Previously Presented) The pharmaceutical composition of claim 70, wherein said second anticancer agent is selected from the group consisting of: cisplatin, carboplatin, and oxaliplatin.

72. (Previously Presented) The pharmaceutical composition of claim 71, wherein said second anticancer agent is carboplatin.

73. (Previously Presented) The pharmaceutical composition of claim 38, comprising a second anticancer agent.

74. (Previously Presented) The pharmaceutical composition of claim 73, wherein said second anticancer agent is selected from the group consisting of: cisplatin, carboplatin, and oxaliplatin.

75. (Previously Presented) The pharmaceutical composition of claim 74, wherein said second anticancer agent is carboplatin.

76. (Previously Presented) The method of claim 1, wherein said tumor is a breast cancer, ovarian cancer, lung carcinoma, renal carcinoma, or bladder carcinoma.

APPENDIX II

Table 1. Effect of Taxol +/- CA4P on CaNT Tumor-Bearing Mice

Taxol (30 mg/kg)			**CA4P** (50mg/kg)			**Taxol** (30mg/kg) + **CA4P** (50mg/kg)		
Expt.	S.F. /g	± S.E.	Expt.	S.F. /g	± S.E.	Expt.	S.F. /g	± S.E.
SX78	0.4862		SA5	0.0638		SX78	0.0372	
SX80	0.2528		SA8	0.106		SX78	0.0190	
SX80	0.3333		SA31	0.3		SX78	0.0138	
			SA31	0.19				
			SX47	0.144				
			SX48	0.107				
			SX49	0.118				
			SX50	0.129				
			SX51	0.063				
			SX52	0.116				
Mean	**0.3574**	0.0684	**Mean**	**0.134**	0.022	**Mean**	**0.0233**	0.0071